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Morphological and Morphometric Analysis of Paraspinal and Intercostal Musculature on Adolescent Idiopathic Scoliosis

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Abstract

Background: Muscles of the human paraspinal region have been the focus of attention in patients with Adolescent Idiopathic Scoliosis (AIS). Despite the description of differences in muscle histology on the two sides of the scoliotic curve, there is no consensus on the significance of such findings. The purpose of this study was to assess changes in the thoracic wall muscles, including the paraspinal and the intercostal muscles (not previously described in the literature) in patients with AIS, and examine their relationship to the curvature.

Methods: The paraspinal and external intercostal muscles on both sides of the scoliotic curve were biopsied in 29 patients with AIS. Tissues were studied under light microscopy and submitted to morphometric examination.

Results: Both muscles on the concave side of the curve were characterized by a greater percentage of type 2 (fast twitch) fibers and a smaller mean fiber diameter than the contralateral muscles. Fiber diameter in the concavity, for both muscles, showed an inverse relationship to the Cobb angle.

Conclusion: Patients with AIS present variations in the microstructure of both paraspinal and intercostals muscles, with similar changes. This suggests that the process of AIS is broadly based; it affects more than one muscle group in the thoracic wall, and is not confined to the paraspinal region. The new findings on the intercostal muscles may be related to the rotation component of the scoliotic curve. Results suggest the possibility that an imbalance between muscle and vertebral growth may lead to the deformity through a bowstring phenomenon.

Keywords: Adolescent idiopathic scoliosis; Muscle; Paraspinal; Intercostal; Morphology; Morphometry

Introduction

Adolescent Idiopathic Scoliosis (AIS) is considered as a multifactorial syndrome whose etiology involves the interaction of environmental factors and multiple systems of the human body. However, there is still no consensus on the specific role that each factor plays in the origin and development of the deformity [1,2]. It is largely known that many types of myopathies or conditions that affect the function of the muscles are associated with secondary scoliosis [3]. The fundamental role of the thoracic wall musculature on spinal maintenance, support and function makes this system a factor that may also be involved in the etiopathogenesis of AIS [2].

Since the early 1970s, studies describing morphological, morphometric and ultrastructural features of muscles in AIS have been conducted, with special focus on the paraspinal musculature [4-9]. Those aspects remain obscure for other muscles groups that may also influence the origin and development of AIS, such as intercostal muscles, which play an active role in trunk rotation [10,11].

With the aim of further characterizing muscles in AIS beyond the paraspinal region, we analyzed biopsies of the paraspinal and intercostal muscles collected from the same patients during surgical correction of a scoliotic curvature. The muscles from both sides of the curve were compared to verify morphological and morphometric differences between them, and a putative correlation to the Cobb angle. This is the first study, to our knowledge, that evaluates AIS-related disorders in intercostal muscles.

Material and Methods

Patients

From July 2011 through September 2012, 29 girls between the ages of 12 and 18 years of age (average 14.41) underwent surgical procedures aimed at correcting their scoliosis. The majority of the patients had thoracic scoliosis (n=16). There were also patients presenting thoracolumbar (n=9) and double-curve scoliosis (n=4). Twenty patients had used braces prior to surgery, for periods ranging from 3 months to 4 years; 9 patients have never used any such device. Only one patient developed a left-convex deformity. The Cobb angle of the main curve in the patient group varied from 45º to 90º (average 63.66º) (Table 1).

Biopsies

During the surgical procedure, the external intercostal and paraspinal muscles were biopsied (on both sides of the Cobb angle), prior to muscle stripping for the arthrodesis. The biopsies were performed at the apex of the curve at a distance of 5.0 cm and 2.0 cm to the side of the spinous process of the reference vertebra, for both the external intercostal and paraspinal muscles, respectively. During the removal of bone graft for arthrodesis, a sample of the right gluteus maximums was taken as a control for each patient, to exclude myopathy as a cause of scoliosis. All patients gave informed consent and the study was approved by the institutional review board.

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Parameter	Patients (n=29)
Age (years - in surgery)	14.41+1.78 (12-18)
Body mass (kg)	52.33+8.01 (38.4-71.9)
Height (m)	1.60+0.77 (1.47-1.79)
Body mass index (kg/m^2)	20.45+3.76 (15-33.27)
Cobb Angle (degrees)	63.66+12.17 (45-90)

Table 1: Clinical characteristics of patients (mean + SD, range in parentheses).

Morphological analysis

A total of 145 muscle biopsies were obtained. Each biopsy was typically >3 mm in every dimension. The samples were processed according to the description by Dubowitz and Sewry [3]. Sections were stained using the techniques of Hematoxylin and Eosin (HE), Gomori Trichrome and ATPase histochemistry (at pH 9.4). The following features were evaluated: (1) necrosis of muscle fibers, (2) phagocytosis of the necrotic fibers, (3) regeneration of fibers, (4) atrophy, (5) selective atrophy, (6) hypertrophy and (7) fiber-type grouping.

Fiber atrophy was graded as mild, moderate or severe. Mild atrophy was defined as few, small, isolated fibers (Figure 1); moderate atrophy as numerous small fibers, not predominant in a sample; and severe atrophy as small fibers outnumbering larger fibers. We identified selective atrophy of fibers when the smaller fibers were all one type, either type 1 or type 2. Fiber-type grouping was defined by the appearance of fibers that were surrounded only by fibers of the same type.

Gluteus samples were used as a control to verify morphological criteria for myopathy. The comparison was conducted using only the values obtained for paraspinal and intercostal samples (116 samples).

Morphometric analysis

For the morphometric study, the sections reacted for ATPase activity were scanned and photographed at 100x magnification. Analysis was performed using Image Pro® Plus 5.0 (Media Cybernetics, Inc.), to obtain the minimum diameter of each fiber in a given transverse section and the proportion of fast and slow types of fibers. Muscle fibers were classified as type 1 (slow twitch – light fibers) and type 2 (fast twitch – dark fibers) according to the intensity of the stain's color reaction for ATPase 9.4 activity. The evaluation was performed on 100 fibers of each type. The fiber-type proportion was established by counting all of the fibers (at least 100 fibers) for each sample, in a field photographed at 100x.

Statistical analysis

A descriptive statistical analysis of the data was conducted, using standard deviation and confidence intervals to attain the average. The Paired Student t-Test was applied for comparison of the means between the convex and concave sides of the curve, for each parameter. The relation between the morphometric parameters and the Cobb angle was evaluated using Spearman correlations. Significance was established at 5% ($p<0.05$).

Results

Morphological analysis and fiber typing

The analysis confirmed that all patients had AIS, since there were not enough morphological criteria for a diagnosis of myopathy in the gluteus maximums, nor in the paraspinal and intercostal samples.

In 56 (48%) samples (including only intercostal and paraspinal muscle biopsies) the tissue was considered morphologically normal, and in 58 (50%) histological examination revealed mild morphological changes that were considered variations of normality. One sample of intercostal muscle, taken from the curve convexity, was considered inadequate for evaluation and another one presented neurogenic pattern. Necrosis, phagocytosis, hypertrophy and fiber regeneration were observed focally in only a few samples. These minimal morphological alterations were more common in the convexity for both muscles (Table 2).

Fiber–type grouping was found in 51 (44%) samples. Groupings of both types of fibers, type 1 and type 2, were observed. In some samples, both fast and slow fibers showed a grouped distribution in a single section, raising the question of a possible neurogenic disorder. For all muscles evaluated, groups of type 1 fibers were more frequent (29 samples – 25%). Intercostal muscle samples revealed the formation of type 1 (slow twitch) fiber groupings as more common in the convexity (8 samples - 28%), while the formation of type 2 (fast twitch) fiber groupings was more frequent in the concavity (8 samples -28%). Only concavity muscles presented grouping of both types of fibers (3 samples – 10%).

In the paraspinals, grouping of all kinds was more frequent in the concavity, with type 1 grouping the most frequent of them (Table 2).

Morphometric analysis

The fiber diameter measurements for intercostal and paraspinal muscles were compared on the two sides (convex and concave) of the scoliotic curve. Two samples of intercostal muscle from the convexity were inadequate for morphometry as they did not present a sufficient number of fibers (100 of each type); therefore, 114 samples were analyzed for changes in fiber diameter.

The average of the smallest diameter for each type of fiber (1 and 2) was calculated and compared. Type 1 fibers in the intercostal muscles (Figure 2) were significantly smaller on the concave side (37 ± 8.8 μm) than on the convex side (48 ± 7.3 μm) ($p<.0.001$). The same relationship was found with type 2 fibers; 36 ± 6.6 μm in the concavity and 41 ± 7.9 μm in the convexity ($p<0.002$). For the paraspinal muscles, the type 1 fibers were significantly smaller on the concave side of the curve (Figure 3) ($p<0.002$).

For both, intercostal and paraspinal muscles, there were more type 2 fibers on the concave side than on the convex side (Figures 4 and 5).

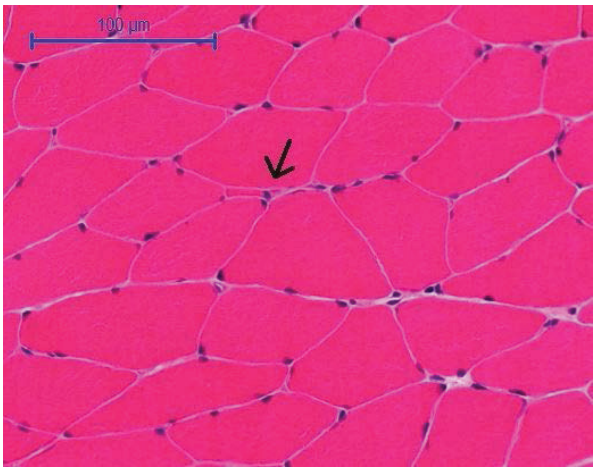

Figure 1: Light micrograph of paraspinal muscle on the concavity (HE). Arrow indicates small atrophic fiber.

Muscle	Necr.		Phag.		Reg.		Atrophy		Select. A.		Hypertr.		Grouping								Final Diagnosis					
													Total		Type 1		Type 2		Both		Normal		Min. Alt.		Other	
IC CC	1	3%	1	3%	1	3%	15	52%	0	0%	2	7%	12	41%	1	3%	8	28%	3	10%	16	55%	13	45%	0	0%
IC CX	3	10%	1	3%	3	10%	16	55%	0	0%	3	10%	8	28%	8	28%	1	3%	0	0%	11	38%	16	55%	2	7%
PS CC	0	0%	0	0%	1	3%	16	55%	1	3%	5	17%	20	69%	12	41%	2	7%	6	21%	16	55%	13	45%	0	0%
PS CX	3	10%	0	0%	0	0%	17	59%	1	3%	2	7%	11	38%	8	28%	1	3%	3	10%	13	45%	16	55%	0	0%
TOTAL	7	6%	2	2%	5	4%	64	55%	2	2%	12	10%	51	44%	29	25%	12	10%	12	10%	56	48%	58	50%	2	2%

Abbreviations: IC CC: Intercostal of the Concavity; IC CX: Intercostal of the Convexity; PS CC: Paraspinal of the Concavity; PS CX: Paraspinal of the Convexity; Necr.: Necrosis; Phag.: Phagocytosis; Reg.: Regeneration; Selec.A.: Selective Atrophy; Hypertr.: Hypertrophy; Min. Alt.: Minimal Alterations.

Table 2: Morphological features of intercostal (IC) and paraspinal (PS) muscles by group on the concave (CC) and convex (CX) side of the scoliotic curve, compiled for all patients, showing the absolute values and percentage for each parameter.

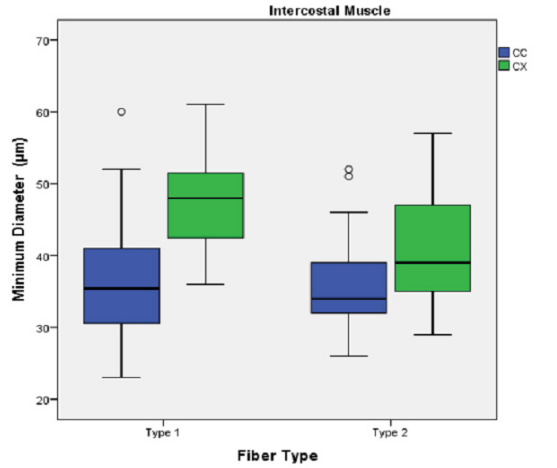

Figure 2: Average diameter of the intercostal muscle fibers (o: outliers).

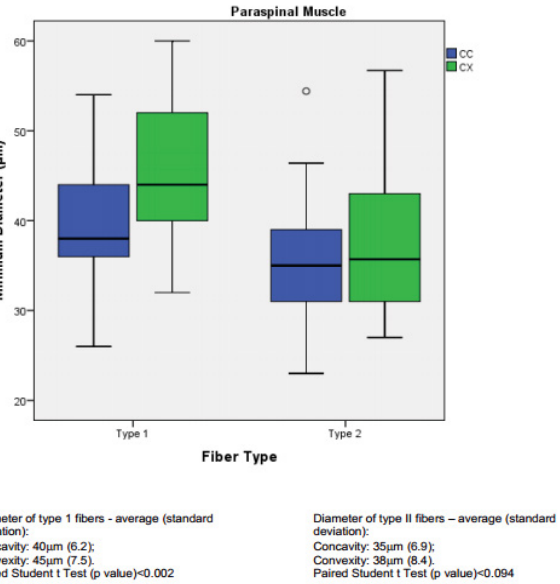

Figure 3: Average diameter of the paraspinal muscle fibers (o: outliers).

Fiber diameter data were significantly correlated with the Cobb angle values. The evaluation showed an inverse relationship: the diameter of both type 1 and type 2 fibers in the concavity of paraspinal muscles decreased as the Cobb angle increased (Figure 6A and 6B). The same relationship was found for type 2 fibers of intercostal muscles in the concavity of the scoliotic curve (Figure 6C).

It bears mentioning that we found no difference between patients who used braces and those who did not.

Discussion

As has been previously pointed out, Adolescent Idiopathic Scoliosis (AIS) is a multifactorial syndrome that clearly involves the skeletal muscles of the thoracic wall. However, while several studies point to the existence of morphological disorders of the paraspinal muscles in patients with AIS [6-8], there is still controversy about whether these disorders cause the scoliotic curve and its progression, or if they are simply secondary abnormalities. Until now, evaluation of muscles in patients with scoliosis was mainly limited to the paraspinal muscles, and did not take into account other muscles of the thoracic wall that could also influence the process. The overarching goal of this study was to analyze the external intercostal muscles, since they play an active role in trunk rotation [10,11], which is an important element in the development of scoliosis.

It was interesting to note that both paraspinal and intercostal muscles showed groupings of both types of fibers in the same samples. This finding suggests a disorder in local enervation, since the presence of fiber groupings of both types is indicative of a de-enervation-and-re-enervation process. Patients with AIS may present changes in the neuroaxis that are characterized by finding the cerebellar tonsils in a lower position relative to the basion-opisthion line [12]. Together with the change in the tonsillar position, the observation of fiber-type groupings for both types of fibers raises the possibility that neurological disorders play a role in the development of AIS.

Fiber diameter was diminished in both intercostal and paraspinal muscles on the concave side of the curve. The occurrence of a smaller minimum diameter of the fibers in the concavity of the scoliotic curve, and its association with a greater percentage of type 2 (fast twitch) fibers in paraspinal muscles is in agreement with data reported by other authors [4,6-9]. In 1979, Yarom et al. [4] showed that patients with different forms of scoliosis had smaller diameter fibers in the paraspinal muscles on the concave side of the curve than those found in the convex side. In 1998, Mannion [8] demonstrated that type IIA fibers (fast oxidative-glycolytic) on the concave side of the curve were significantly thinner than in the control patients. In our patients, only type 1 (slow twitch) fibers in the paraspinal muscles varied significantly in diameter between the two sides of the curve, with the concave side having the smaller fibers.

However, we demonstrated that these differences are not restricted to paraspinal muscles. Furthermore, the changes in fiber diameter related to muscles in the concavity of the curve were more obvious

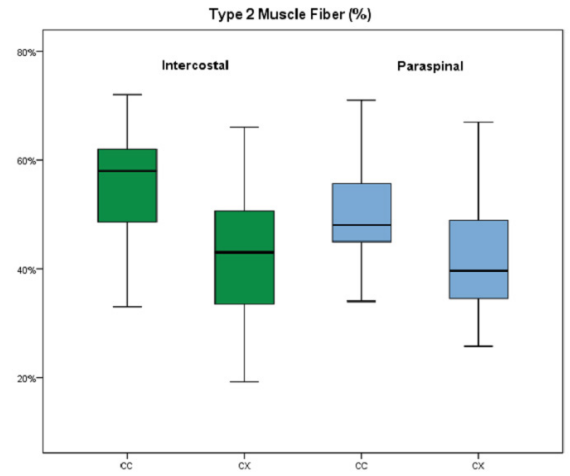

Figure 4: Percentage of type 2 fibers in the intercostal and paraspinal muscles on the concave (CC) and convex (CX) side of the scoliotic curve.

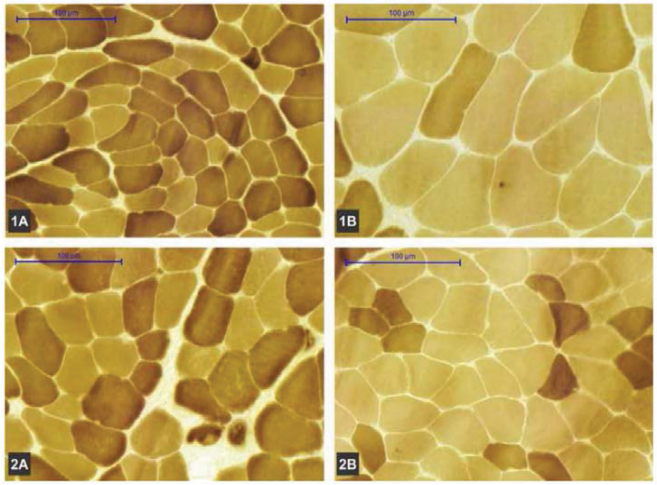

Figure 5: Light micrograph of intercostal and paravertebral muscles (ATPase 9.4-type 1 fibers lighter and type 2 darker). Intercostal muscles concave (1A) and convex (1B) and paraspinal muscles concave (2A) and convex (2B).

in the intercostal muscles. According to a previous study, the average fiber diameter of the external intercostal muscle fibers in normal adult women is about 46 ± 6.8 μm for type 1 fibers and 43.7 ± 7.1 μm for type 2 fibers [13]. In our study, the average diameters in the convexity were similar to these values, while in the concavity they were 37 μm for type 1 fibers and 36 μm for type 2. It demonstrates that, in a similar way to paraspinal muscles, intercostal muscles atrophy in the concave side of the curve. These changes confirm the clinical-surgical impression that thoracic wall structures are widely involved in AIS.

We can speculate that the small-diameter fibers on the concave side of the scoliotic curve could be the result of a reduction in the physical space available for growth; such a restriction could impede growth and prevent full use of the muscles. The inverse correlation between fiber diameter and the Cobb angle provides further support for this idea, although it was observed only for type 2 fibers in intercostal muscles and for both type 1 and type 2 fibers in the paraspinal muscles. Consistent with this idea, one would anticipate that type 1 fibers of intercostal muscles would eventually be involved in this inverse correlation with the Cobb angle, since the physical space becomes more restricted during progression of AIS; this is a testable prediction.

On the other hand, the smaller diameter of fibers could be due to the reduced capacity for growth of the muscles on the concave side of the curve. It is well established that disuse of muscle fibers has an impact on the ability of satellite cells to become activated for proliferation and growth. This is can be identified in conditions such as denervation or age-related atrophy. For example, with increasing age, satellite cells in mouse muscles become increasingly refractory to activation by stretching in culture [14]. At least in mice, this accompanies the development of sarcopenia and also limits the capacity for muscle growth through exercise [15].

It is possible that in AIS, there is an imbalance between muscle satellite cell activity and muscle growth, and skeletal (vertebral) growth; this imbalance may lead to the deformity of spinal curvature, inducing a "bowstring" phenomenon in shorter or atrophic muscles spanning the intervertebral joints on the concave side of the curve. It would be interesting to determine whether the level of satellite cell activation or its response to stretching differs on the concave and convex side of a scoliotic curve.

However, it is certain that immobilization (i.e., in this case, of particular segments or regions of the thoracic wall) is an important cause of atrophy. Restricted movement and disuse may be responsible for the variations we observed, and the laterality of those variations. In the case of the intercostal muscles, this might be a consequence of the limitations in trunk movement per se and also from the increased breathing difficulty observed in patients with more severe deformity.

With regards to fiber-type proportions, a study about respiratory muscles [16] reported that the intercostal muscles, both internal and external, have over 60% type 1 (slow twitch) fibers. Reports on paraspinal muscles indicate a similar predominance of type 1 fibers (almost 70%) that is a characteristic of this musculature, especially in women [17]. Our results show a significant change in this feature, and point to a relatively greater proportion of type 2 (fast twitch) fibers on the concave side of the curve in patients with AIS. On the convex side, the proportion is closer to that described in the literature for normal, non-AIS muscles. In view of these findings, the increased percentage of type 2 fibers on the concave side of the curve suggests greater anaerobic and glycolytic activity. Such metabolic adaptations are associated with more frequent high-intensity activity of this type of fiber rather than the development of low-intensity contraction and fatigue resistance in the muscles on the concave side. This may be related to the constant attempts at compensatory postural adjustment [18,19], as well as the increased respiratory effort of patients with more severe scoliosis.

Genetic studies on myosin heavy chains [20] show that immobilization leads to significant reduction in the proportion of type 1 (slow twitch) fibers, in addition to causing atrophy in both type 1 and 2 fibers. Immobilization also decreases the level of satellite cell activation, essential for the development of new and larger muscle fibers [14]. The putative reduced use of muscles on the concave side in relation to the convex side of the scoliotic curve could, then, explain both the decrease in fiber diameter as well as the greater percentage of type 2 (fast twitch) fibers on the concave side.

It is interesting to speculate whether the curvature of the spine is primary or secondary to rotation of the trunk. In other words: does the rotation serve as a primary trigger of the curvature or is it the other way, that is, does the curvature trigger secondary rotation? This question emerged from the current findings showing that the

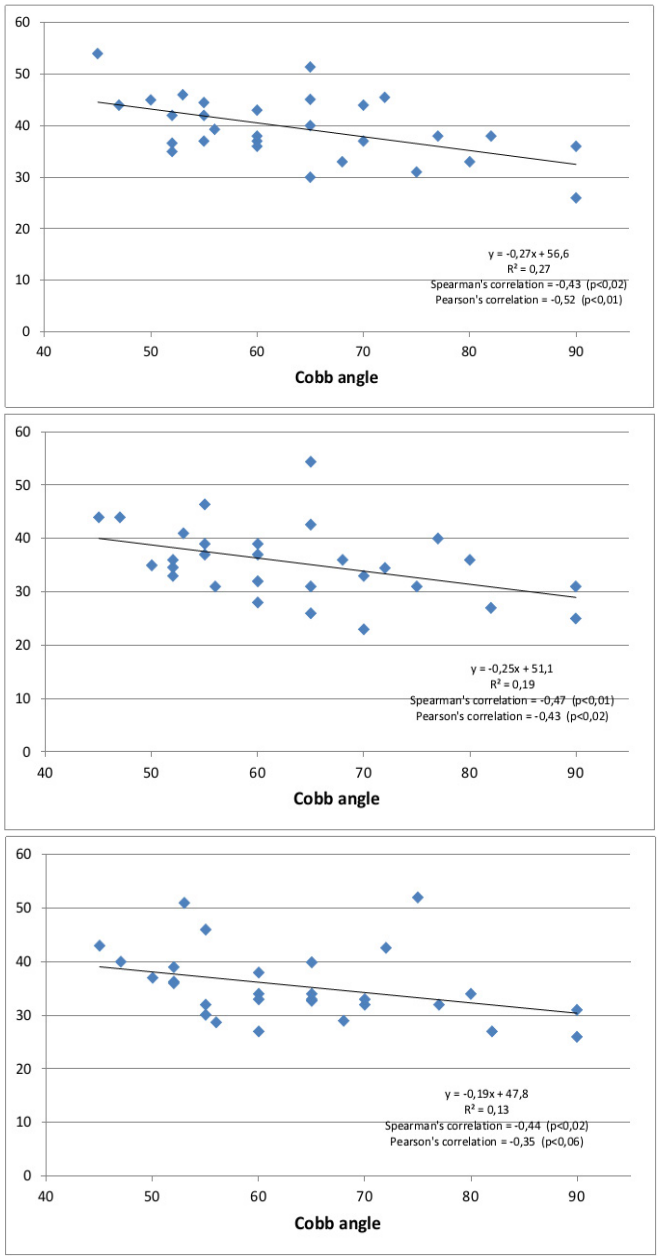

Figure 6: Correlation studies of fiber diameter (y-axis in μm) and Cobb Angle (x-axis in degrees). (A) Paraspinal muscles from the concavity, type 1 fibers. (B) Paraspinal muscles from the concavity, type 2 fibers. (C) Intercostal muscles from the concavity, type 2 fibers.

intercostal muscles are also disrupted in scoliosis. As indicated earlier, the intercostal group of respiratory muscles also participates in trunk-rotation movements. Further studies should be conducted to evaluate this possibility.

Conclusion

Our findings in this study lead us to infer that, in AIS, some features of the thoracic wall muscles can be characterized by both fiber atrophy and an increased percentage of type 2 (fast twitch) fibers on the concave side of the scoliotic curve. These features have been found in the paraspinal muscles, as previously reported, and also in the intercostals, demonstrating that AIS is a broadly-based process. Findings about the intercostal muscles also raise questions about mechanical aspects, e.g., rotational components.

Nevertheless, the possibility of a causative imbalance between muscle strength and use; fiber satellite cell activity and activation; and fiber and/or partial muscle denervation is still to be established. A possible imbalance between muscle and vertebral growth, leading to the deformity through a bowstring phenomenon, should also be considered.

Finally, further investigation is needed to verify whether 1) the changes described in this study are a cause or a consequence of the deformity or of the progression of AIS; and 2) whether and how other factors might also influence the origins of this syndrome.

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